

Human insulin and porcine insulin in the treatment of diabetic children: comparison of metabolic control and insulin antibody production

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Abstract

Semisynthetic human insulin and highly purified porcine insulin were compared in a double blind crossover study in 21 diabetic children. Glycosylated haemoglobin values at the end of four month treatment periods were higher after treatment with human insulin than after treatment with porcine insulin (mean 15.7% (SD 2.3%) v 14.2% (2.3%); $p < 0.01$). Higher fasting blood glucose concentrations occurred during treatment with human insulin than with porcine insulin (mean 12.0 (SD 2.1) v 11.0 (2.4) mmol/l; mean 216 (SD 38) v 198 (43) mg/100 ml; $p < 0.05$), but there were no significant differences at other time points during the day. The incidence of hypoglycaemia was similar for both treatment groups. Concentrations of antibody reactive with porcine and human insulins were similar for the two treatment groups, although greater fluctuation was observed in the amount of antibody reactive with human insulin.

Semisynthetic human insulin is safe and effective in diabetic children, although further work is needed to devise regimens which achieve optimal blood glucose control.

Introduction

Although human insulin differs from porcine insulin by only one amino acid situated at the C terminal of the B chain,¹ its potential benefits have excited great interest. Human insulin is now available for clinical use and is currently prepared by enzymatic semisynthesis from porcine insulin²⁻³ or by recombinant DNA techniques⁴ using *Escherichia coli*.

Studies of semisynthetic human insulin show that it is safe in normal adults⁵ and, when using the glucose clamp technique, that its potency is indistinguishable from that of porcine insulin.⁶ Similar free insulin profiles have been recorded in adult diabetics after injection of porcine and human insulins.⁷ We have compared semisynthetic human insulin with highly purified porcine insulin in diabetic children and have assessed clinical, metabolic, and immunological variables.

Patients and methods

Twenty one children aged 5-16 years who had had a history of diabetes for two to 12 years were recruited from a paediatric diabetic clinic. All were taking twice daily short and intermediate acting insulins and were carrying out home blood glucose monitoring using

BM-Test-Glycemic 20-800 sticks (Boehringer Corporation Limited). The correlation between blood glucose values monitored in the laboratory and at home was 0.82 ($p < 0.001$) over the range 5.8-18.0 mmol/l (105-324 mg/100 ml). All children were free of diabetic complications other than mild contractures of the interphalangeal joints of the fingers, and those with other chronic disease were excluded. The investigation was explained to their families and approval obtained from the local ethical committee. The children were seen by one investigator throughout.

Nineteen children were using highly purified porcine insulin at entry (18 Velosulin and Insulatard (Nordisk), and one Actrapid and Monotard (Novo)). Two children were using bovine insulin (soluble and isophane) at entry, and 11 other children had previously been exposed to bovine insulin. Results of autoantibody screening were negative in 16 children; islet cell antibodies were present in two, thyroid antibodies in two, and parietal cell antibodies in one. In 17 children serum C peptide was undetectable throughout (< 0.01 nmol/l; < 3 ng/100 ml).

Procedure—The study protocol consisted of a three month run in period taking the original insulin followed by three four month treatment periods using a double blind crossover technique. Description of the groups and time points during the study are given together—for example, group A at month 3 is A3 (fig 1). Treatment

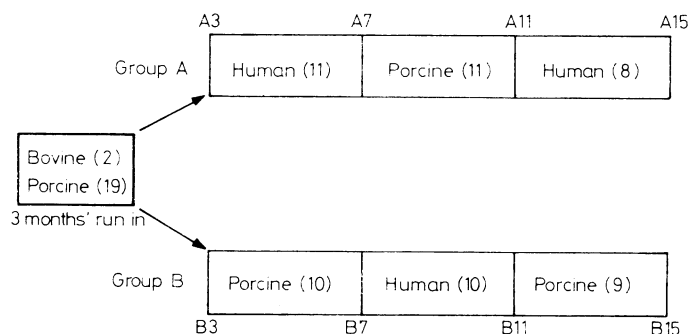


FIG 1—Plan of study, indicating species of insulin used before and after randomisation into groups A and B. The three treatment periods of four months are shown for both groups. Numbers of patients given in parentheses.

periods of four months were used because most of any change in insulin antibody value after crossover takes place during this time.⁸ The children were randomised into two treatment groups, group A being given human insulin and group B porcine insulin in the first four month treatment period. Each group included one of the two children previously treated with bovine insulin. There was no significant difference in age, duration of disease, insulin dose, body mass index, or carbohydrate intake between the two groups (table 1).

TABLE 1—Characteristics of children in groups A and B on entry to study. Figures are means (SD in parentheses)

	Group A (n = 11)	Group B (n = 10)
Age (years)	12.0 (2.3)	11.2 (2.7)
Sex	7M, 4F	7M, 3F
Duration of diabetes (years)	5.2 (1.8)	5.3 (3.7)
Total insulin dose (units/day)	40.4 (17.5)	35.1 (13.1)
Insulin dose (unit/kg/day)	0.88 (0.17)	0.88 (0.12)
Body mass index (kg/m ²)	19.5 (2.0)	17.9 (1.6)
Carbohydrate intake (g/day)	223 (5.4)	204 (3.7)

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Human or porcine soluble (Actrapid) and lente (Monotard) insulins with a proinsulin content below 1 ppm were used in a strength of 40 U/ml during the study. Children were seen on a monthly basis at home but attended the outpatient clinic at each crossover point. The number of hypoglycaemic episodes that had required treatment with extra carbohydrate was recorded at each monthly visit. Four patients left the study at month 11 as they received inappropriate insulins thereafter. Twenty four hour urinary glucose excretion and seven point home monitored blood glucose profiles (before and after meals, and before bed) were determined at monthly intervals. Twice weekly, four point home monitored glucose profiles were used to adjust the dose of insulin. Capillary blood was obtained from finger pricks at monthly intervals for total glycosylated haemoglobin (haemoglobin A_{1c}; HbA_{1c}) estimation. In addition, at each four month visit measurements were made of height and weight as well as C peptide, insulin antibody, and haemoglobin concentrations, total white cell count, erythrocyte sedimentation rate, urea and electrolyte concentrations, liver function values, and cholesterol and triglyceride concentrations.

Laboratory methods—HbA_{1c} was measured by an electroendosmosis method (Corning Medical and Scientific) with an interbatch coefficient of variation of 6.6% (mean HbA_{1c} 13%). Serum C peptide concentration was measured by radioimmunoassay.⁹ Insulin antibody concentrations were measured immunochemically by a second antibody coprecipitation assay.¹⁰ IgG antibody reactive with both ¹²⁵I labelled porcine and human insulins was measured in each sample. Positive and negative quality control sera were incorporated into each assay batch and all tests were performed in duplicate. A normal serum binding value was not subtracted from the test data.¹¹

Analysis of data—Differences in HbA_{1c} value, 24 hour urinary glucose excretion, and blood glucose profiles during treatment with human and porcine insulins were compared by pooling the results from groups A and B (fig 1) after periods of treatment with the same insulin. Results are expressed as means (SD) unless otherwise stated. Paired and unpaired data were compared using Student's *t* test (two tailed). Only 17 pairs of results were available at month 15, as four patients left the study at month 11. The frequency of hypoglycaemia during treatment with the two insulins was compared by Wilcoxon's signed rank test.

Results

No adverse reactions were experienced during treatment with human or porcine insulin. Dosage of the two insulins was closely similar—0.92 unit/kg/day (human), 0.93 unit/kg/day (porcine). The proportions of intermediate to total insulin dose ranged from 56% to 60% with no difference between human and porcine insulin treatment periods.

HbA_{1c} values at the end of treatment periods were higher after the use of human insulin (A7 with B11; fig 1) than after the use of porcine insulin (B7 with A11)—15.7% (2.3%) *v* 14.2% (2.3%); *p* < 0.01. Mean HbA_{1c} values were also higher when the patients were treated with human insulin compared with porcine insulin during months 7–15 (table II).

TABLE II—Comparison of means (SD) of four consecutive monthly glycosylated haemoglobin (HbA_{1c}) values during treatment periods with human and porcine insulins

	Human insulin A11-A15/B7-B11	Porcine insulin A7-A11/B11-B15	Significance of difference
Mean percentage HbA _{1c} * (17 patients)	14.4 (1.8)	13.8 (1.7)	0.05 <i>p</i> > 0.02

*Data at start of each treatment period—that is, A7, B7, A11, B11—not included.

Blood glucose profiles determined during months 3–11 disclosed a higher fasting glucose concentration with human than porcine insulin treatment—12.0 (2.1) *v* 11.0 (2.4) mmol/l; 216 (38) *v* 198 (43) mg/100 ml (*p* < 0.05) (fig 2). The slightly higher glucose values during treatment with human insulin throughout the rest of the day were not significantly different at any other time point. The frequency of hypoglycaemic episodes requiring treatment was 0.8 and 0.3/month with human and porcine insulins, respectively (NS).

Twenty four hour urinary glucose excretion calculated as mean values over each treatment period was similar with human and porcine insulin between months 3 and 11—268 (181) mmol/day *v* 259 (192) mmol/day (48.3 (32.6) g/day *v* 46.7 (34.6) g/day) (NS)—and between months 7 and 15—282 (192) mmol/day *v* 301 (141) mmol/day (50.8 (34.6) g/day *v* 54.2 (25.4) g/day) (NS).

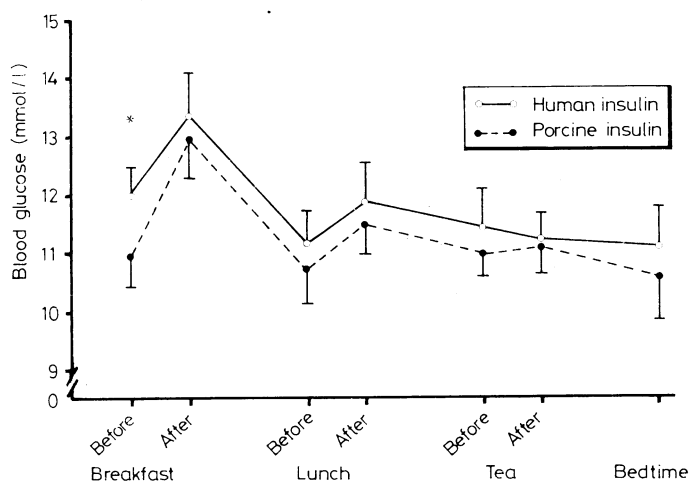


FIG 2—Mean of 84 blood glucose observations at each of seven time points during days when children were receiving human or porcine insulins, respectively. Bars are SEM.

**p* < 0.05.

Conversion: SI to traditional units—Glucose: 1 mmol/l \approx 18 mg/100 ml.

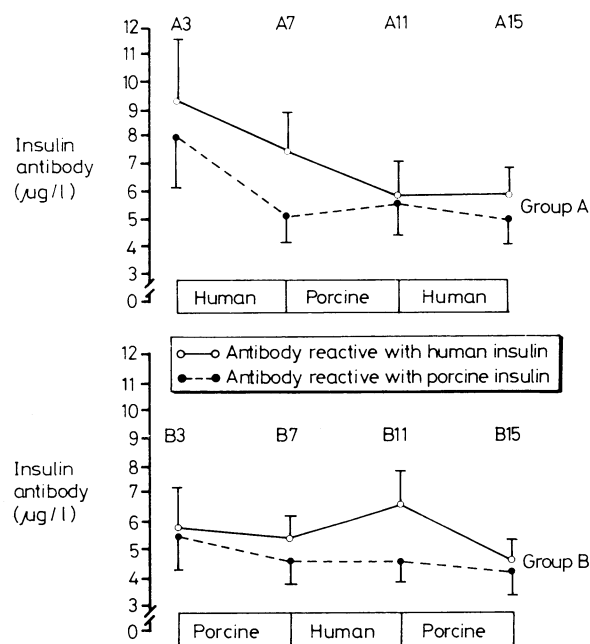


FIG 3—Insulin antibody (µg/l) reactive with human and porcine insulins in groups A and B throughout study. Values are means. Bars are SEM.

Sequential insulin antibody results showed an overall fall in antibody concentrations for both groups A and B (fig 3) throughout the study. Patients in group A did, however, show rather higher concentrations of antibody at the end of the run in period (A3) compared with group B (B3) and a greater degree of binding for human compared with porcine insulin. Although a similar disparity developed in group B during the treatment period with human insulin, both groups showed comparable levels of binding at the conclusion of the study. Antibody reactive with porcine insulin fluctuated much less than antibody reactive with human insulin, and

greater disparity in the binding of human versus porcine insulin occurred at the end of each treatment period with human insulin in contrast to the levels observed at the end of treatment periods with porcine insulin. There was a reduction in the levels of binding to human insulin during treatment with porcine insulin (A7 v A11, and B11 v B15)—7.6 (4.7) v 6.1 (3.5) $\mu\text{g l}^{-1}$ ($p < 0.05$), and 6.7 (4.1) v 4.4 (2.1) $\mu\text{g l}^{-1}$ ($p < 0.05$), respectively.

Measurements of full blood count, liver function values, and urea, electrolyte, cholesterol, and triglyceride concentrations remained unchanged and within normal limits throughout.

Discussion

It is notoriously difficult to achieve adequate blood glucose control in diabetic children.¹² Since they are at high risk of microvascular disease¹³ and have decades of treatment ahead of them, the potential benefits of a new treatment need to be assessed critically. The immunological responsiveness of children to insulin may differ from that of adults, further strengthening the case for a separate study.^{14, 15} Our study was designed so that each child had two crossovers of insulin species prepared in highly purified soluble and lente forms.

Most of the children participating in this study had poor diabetic control as judged by HbA_{1c} values, but these tended to be higher during treatment with human compared with porcine insulin. Fasting blood glucose concentrations were higher with human insulin and similar results have been observed in patients treated with biosynthetic human insulin.¹⁶ This may be due to a difference in the pharmacokinetics of these two insulins, with a slightly accelerated absorption of human compared with porcine insulin, possibly associated with a shorter duration of action.¹⁷ Other studies using the glucose clamp technique or glucose controlled infusion system (artificial pancreas), however, have failed to detect these small differences.^{6, 18} During this study no attempt was made to alter the ratio of short to intermediate acting insulin, which remained constant throughout.

Concentrations of insulin antibody reactive with both human and porcine insulin were measured at each change of insulin species. Comparable levels of binding are usually found for both these ligands in patients treated with bovine, porcine, or human insulins.¹¹ In our study these levels were broadly similar, although patients in both groups showed a mild preference for binding to human insulin; this difference was present in group A on entry to the study (fig 3). This preferential binding may be related to previous exposure to a variety of bovine and porcine insulin preparations.

Insulin antibody reactive with both species of labelled insulin showed an overall fall throughout the study and, although group A started out with higher binding levels, the levels were comparable for both groups at the end of the study, both groups showing comparable binding for the two ligands. A greater degree of binding of human versus porcine insulin was evident at the end of each human insulin treatment period compared with the levels observed at the end of each porcine insulin treatment period. A larger study in adults using a similar protocol has not shown such differences in insulin antibody production during porcine and human insulin treatment (P D Home, N P Mann, A S Hutchinson, *et al*, submitted for publication), but possibly children may respond more vigorously to minor changes in insulin chemistry or formulation. It is unlikely, however, that the changes observed in the present study are of clinical significance. A study in newly diagnosed diabetics suggested that there is less antibody production to human insulin compared with porcine insulin.¹⁹ Administration of homologous—that is, prepared from the same species—insulin has been shown to induce antibodies in man as well as other animals²⁰ and, although the mechanism is not fully understood, probably it includes some form of physicochemical change occurring either before or after injection.²¹

The human and porcine insulin preparations used were effectively free of proinsulin and had a desamido insulin content of $<0.2\%$ at the time of manufacture (J Brange, unpublished

observations, 1983). As they were both of similar age, it is unlikely that significant differences in desamido content would have occurred by the time of administration.

We have shown that semisynthetic human insulin is a safe and effective preparation in children with established diabetes. Further work is needed to determine optimal regimens for human insulins, as a shorter duration of action may necessitate alteration in the proportions of short and intermediate acting insulins²² as well as the timing of the evening insulin injection. At present there does not appear to be a case for routine transfer of diabetic children from highly purified porcine to human insulin.

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